PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 02-1037-I)

In the Application of:	)	
Jeffrey D. Hillman	)	
	)	**Examiner: Ware**
Serial No.: 10/567,592	)	
Filing Date: February 6, 2006	)	
	)	**Group Art Unit: 1651**
U.S. Nat'l Phase of PCT/US2004/025899	)	
Intn'l Filing Date: 10 August 2004	)	
	)	**Conf. No.: 1322**
For: Compositions and Methods for the Maintenance of Oral Health	)	

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

1. I, Dr. Robert Zahradnik, founded Oragenics, Inc., the entire assignee of the above-mentioned patent application, in 1996, along with Dr. Jeffrey Hillman. I have served in several capacities at Oragenics, Inc. since 1996, including Director, President, Chief Operating Officer, and VP of Product Development. I currently serve as the Vice-President Product Development for Oragenics, Inc. My past forty years have been spent in varying aspects of the dental research field, both at publically-funded institutes and at private and public dental product companies. Prior to joining Oragenics full time in 2005, I was a Director of iviGene Corporation, Alachua, Florida, a company engaged in the business of developing vaccines and biomarkers. Previously, I served as General Manager of ProHealth, Inc. in Batesville, Arkansas, a Partner and General Manager of Professional Dental Technologies and Therapeutics, Batesville, Arkansas, an oral care pharmaceutical manufacturer, and the Chief Executive Officer of Advanced Clinical Technologies, Inc. Medfield, Massachusetts, a dental diagnostics manufacturer and technical consulting firm. From June 1979 to February 1986, I was engaged as a senior scientist and project manager at Johnson & Johnson Oral Care, Windsor, New Jersey and then at the J&J's Cambridge Biotech Research Laboratories in Cambridge, Massachusetts. From June 1971 until January 1979, I was a research scientist at the Harvard-affiliated Forsyth

Institute focused on basic research into the causes and treatments of oral diseases. I graduated from Pennsylvania State University with a Bachelor of Science degree in Chemistry and from Boston University with a Ph.D. in Physical Chemistry. A copy of my curriculum vitae is filed herewith.

2. The claimed combination of isolated bacterial strains in the above-mentioned application provides an unexpected improvement or effect over the prior art. It has been discovered that the claimed combination of bacterial strains unexpectedly promotes tooth whitening along with maintaining oral health (e.g., treatment, prevention or both treatment and prevention of periodontitis, dental caries, *Candida* or fungal overgrowth, halitosis, xerostomia-induced dental caries or periodontal disease, oral bacterial infections, oral bacterial disease, oral wounds or a combination thereof).

3. Stained dental ceramic material, representative of stained dental enamel, was treated with a suspension of *S. oralis* under my supervision at Oragenics, Inc. to determine if *S. oralis* can promote tooth whitening. These whitening experiments followed standard laboratory models for evaluating cleaning/whitening capacity of dental products. *See e.g.*, Sharif *et al.*, British Dent. J. 188:620 (2000) (copy of abstract attached); Lath *et al.*, Internat. J. Dent. Hyg. 4:129 (2006) (copy of abstract attached); Moore *et al.*, BMC Oral Health. 8:23 (2008)(copy attached). A suspension of *S. oralis* was incubated *in vitro* in the presence of glucose and oxygen, to determine if *S. oralis* could produce sufficient hydrogen peroxide to produce a measurable whitening effect on stained ceramic disks resembling teeth.

4. Ten dental ceramic disks were stained over an eight-week period with tea (Lipton) and chlorhexidine (0.12%, Hi Tech Phamacal Co., Amityville, NY). Each disk was placed in a 50 ml conical (Falcon) plastic test tube. Three ml of brewed Lipton tea (prepared by the addition of a family size Lipton tea bag to 200 ml of boiling water for 5 min) was added to cover the disks. After 24 hr incubation at room temperature, the tea was removed by decanting, the disks were rinsed with 5 ml of tap water, and the tea solution was replaced with 3 ml of 0.12% chlorhexidine for 24 hrs. Steps 2 and 3 were repeated for 4 weeks, Monday through Friday, and the disks remained in Friday's solution over the weekend. The lightness of the disks was quantitatively measured using a Chroma Meter CR-400 colorimeter (Minolta, Ramsey, NJ). Lightness values for the disks were generated directly by placing the instrument's measuring head over the disks. Standard color plates were used to calibrate the colorimeter.

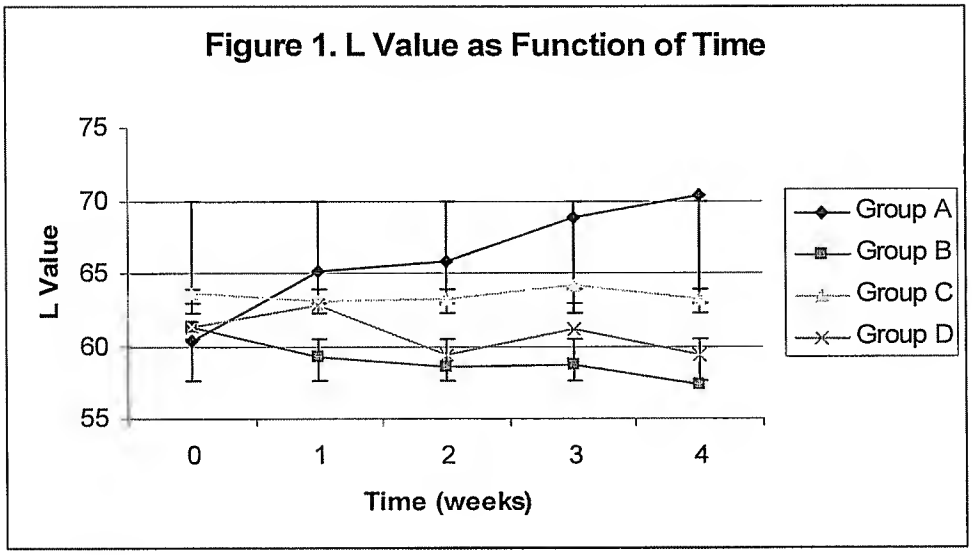
5. The treatment phase began one day after the final colorimeter readings were taken. Nine separate cultures of *S. oralis*, strain KJ3sm, inoculated from starter plates, were grown in 30 ml of Todd Hewitt Broth (Difco; Bacto

Catalog No. 249240) supplemented with 0.1% sodium bicarbonate/0.5% glucose/1mg/ml streptomycin sulfate in an environmental shaker (200 rpm) at 37°C. After overnight incubation, the cells were harvested by centrifugation at room temperature, washed once with 10 ml of Amies media, and resuspended in 30 ml of Amies media with or without dextrose (glucose; Fisher Scientific, Catalog No. D16) and catalase (Sigma Aldrich Catalog No. C93225G) as shown in Table 1. The control (Group D) contained 30 ml of Amies medium with dextrose and inactivated catalase. Where indicated, catalase was inactivated by heating in a boiling water bath for 5 minutes. The entire 30 ml aliquots described in step 2 and Figure 1 were added to 50 ml Falcon tubes containing 1 stained dental ceramic disk per tube. These treatment steps were repeated daily, Monday through Friday, for 4 weeks, and the disks remained in Friday's solution over the weekend.

Table 1

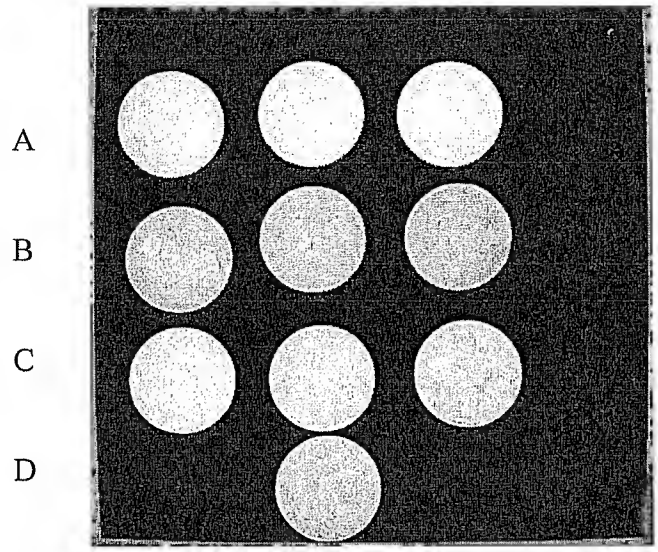
GROUP	DISK NUMBER	TREATMENT CONDITIONS		
		KJ3sm (~10^9 cfu/ml)	Dextrose (0.5%)	Catalase (3000 U/ml)
A[1]	1,2,3	+	+	Inactivated
B[2]	4,5,6	+	+	Active
C[3]	7,8,9	+	-	Inactivated
D[4]	10	-	+	Inactivated

[1] Experimental
[2] Catalase control
[3] Dextrose control
[4] Amies control



6. The lightness value for each disk was measured weekly during the treatment phase, which was carried out over a four-week period. The L value (lightness) was plotted as a function of time using the mean group values (± S.D.; Figure 1). The trendline for Group A data had a slope of 2.37, which was substantially greater than the slopes of the trendlines for Group B (-0.54), Group C (0.52) and Group D (-0.84), indicating that disks in Group A were becoming lighter or whiter over time at a much faster rate than the disks in the other groups. At the conclusion of the 4 week experimental period, a lightening or whitening of the ceramic disks in Group A was readily apparent to the unaided eye (Figure 2).

Figure 2.



7. Inter- and intra-group L values were compared for statistically significant differences. A 4 (Treatments: A, B, C, D) x 5 (Time: Weeks 0, 1, 2, 3, 4) mixed-model repeated measures ANOVA was conducted with Greenhouse-Geisser correction to determined the differential influence of various treatments on L values across time. Follow-up analyses determined treatment effects at each time point. Post hoc Bonferroni-corrected t tests were used to determine significant differences among the treatments. All analyses used a family-wise of $p = .05$. Results indicated significant effects of Treatment, $F(3, 6) = 6.0$, $p < .05$, and Time, $F(1.3, 24) = 12.7$, $p = .005$, which were superseded by a Treatment x Time interaction, $F(4, 24) = 58.7$, $p < .001$. Decomposition of this interaction yielded non-significant findings at baseline (Time 0) and Week 1, $F(3, 6) < 4.4$, $p > .05$. At Weeks 2-4, Treatment Group A yielded a significantly larger effect than Treatments B and D, $t(3) > 8.1$, $p \leq .01$, and a significantly larger effect than Treatment C only during week 4, $t(2) = 4.1$, $p = .015$. No significant differences were observed between Treatments B, C, and D ($p > .05$).

8. *S. oralis* incubated in the presence of glucose and air produced a statistically significant whitening effect on tea and chlorhexidine-stained ceramic disks after 4 weeks of exposure to *S. oralis*. Inclusion of catalase

in the incubation medium significantly reduced any whitening effect, suggesting that the mechanism of whitening involved hydrogen peroxide production by *S. oralis.* Peroxide production, and thus a whitening effect, was dependent on the presence of a metabolizable carbon source, such as glucose. The small whitening effect observed in Group C may be due to residual peroxide production resulting from metabolism of stored carbohydrate (e.g., in the form of intracellular polysaccharide). The plot of L value as a function of time for Group A in Figure 1 did not plateau, indicating that maximum whitening effect had not occurred within the timeframe of the study, and that longer treatment with KJ3sm would likely achieve a greater whitening effect.

9. The claimed combination of bacterial species provides an unexpected improvement or effect over the use of one species in isolation. Although the tooth whitening data refers only to *S. oralis*, I expect with confidence that the data provides a basis to predict that a combination of one or more *S. oralis*, *S. uberis,* and lactate dehydrogenase-deficient *mutans streptococcus* strains provides an advantage over using a single species in isolation. This is because different species of bacteria colonize different surfaces or portions of teeth. Therefore, the use of more than one species of bacteria can be used to "blanket" all or most surfaces of the teeth, whereas the use of only one species of bacteria may result in certain surfaces or portions of the teeth being uncolonized. Hillman *et al.* demonstrates that *S. oralis* (previously known as *S. sanguis*) and *S. mutans* have "physically distinct, non-overlapping niches" on teeth. J. Dent. Res. 66:1092 (1987); *see* page 1094; copy filed herewith.

10. It was unexpected that bacteria, such as *S. oralis* and *S. uberis,* could produce hydrogen peroxide in an amount sufficient to actually whiten teeth along with providing oral health benefits such as the treatment, prevention, or both treatment and prevention of periodontitis, dental caries, *Candida* or fungal overgrowth, halitosis, xerostomia-induced dental caries or periodontal disease, oral bacterial infections, oral bacterial disease, oral wounds or a combination thereof. Therefore, it was unexpected, in light of the prior art, that a combination of *mutans streptococci* strains, wherein the *mutans streptococcus* strains are lactate dehydrogenase-deficient, *S. oralis* strains and *S. uberis* strains could be used to promote oral health and to promote cosmetic appearance by whitening teeth.

11. I declare that all statements made herein to my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States

Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 10/19/10

Signed: Robert T. Zahradnik
Dr. Robert Zahradnik